Research Option Thesis

Optogenetic Manipulation of *Caenorhabditis elegans* Pharyngeal Muscles Inhibit Food Consumption

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Abstract

Obesity, a complex condition that arises from a host of interwoven environmental, cultural, social, and genetic factors, is associated with many devastating diseases. However, resolving the intricacies of obesity-related genes and molecular mechanisms could be especially promising in addressing genetic predispositions to obesity. In mammals, the signaling molecule neuropeptide Y (NPY) is involved in various processes related to obesity, including fat generation, thermogenesis, moderation of feeding behavior, cardiovascular regulation, and much more. By identifying the phenotype of NPY that benefits an organism's fitness, we propose that we can discern why certain alleles of the gene arose in the population, leading to a better idea of potential physiological functions to target in obesity treatments. Conveniently, the simple nematode worm *C. elegans* has a homolog of human NPY receptors which is encoded by the npr-1 gene. This gene, in addition to the *C. elegans* globin protein gene glb-5, has an effect on both fitness and food consumption via unclear, complex mechanisms and potential pleiotropic effects. Considering the theorized links between fitness and genetic predispositions for obesity, these genes could have direct applications to the study of obesity in humans. Here, we design an optogenetics experimental paradigm which will eventually allow us to explore causative connections between food consumption, lifespan, fitness, and reproduction as a result of differences in npr-1 and glb-5 alleles. Using a 90s on/30s off LED cycle, we are able to restrict food consumption *C. elegans*. This process will be the first step in providing insight into potential mechanisms salient to energy metabolism, satiety, and genetic predispositions to obesity.

Introduction

Obesity is a devastating epidemic in the United States which carries a host of co-morbidities and significantly reduces lifespan[1]. As a "complex disease", it arises from a host of interacting environmental, cultural, social, and genetic factors. For complex diseases in general, recent changes in environmental factors could have an effect on the robustness of underlying genetic systems, elevating disease susceptibility[2], making it even more challenging to pin down root causes and corresponding treatments. However, there is a significant level of heritability in obesity cases which suggests the potential use of genetic tools to treat the disease[3]. Many theories propose that certain genes related to metabolic functions and fat storage enhanced fitness in ancestral humans, contributing to the obesity epidemic today[4,5]. One candidate gene is a neuropeptide receptor. In mammals, the signaling molecule neuropeptide Y (NPY) is involved in various processes related to obesity, including fat generation, thermogenesis, moderation of feeding behavior, cardiovascular regulation, and much more[6-14]. However, because NPY has such a broad effect on physiology, determining which of its corresponding phenotypes to target in obesity treatment becomes increasingly challenging. By identifying the phenotype of NPY that benefits an organism's fitness, we propose that we can discern why certain alleles of the gene arose in the population, leading to a better idea of potential physiological functions to target in obesity treatments.

To identify this phenotype, we focus on the nematode model organism *Caenorhabditis elegans*, which is an ideal species with which to study genetics and evolution, given its a short life span, small size, and fully mapped neural connectome. *C. elegans* has a homolog of the mammalian NPY receptor called *npr-1*, which has been shown to influence feeding behavior mediated by aerotaxis, food consumption, size, sexual maturity, and pheromone sensing[15,16]. Interestingly, *C. elegans* worms with the ancestral allele have lower fitness in laboratory conditions compared to worms with a more recently evolved allele[15]. However, while there have been some theories as to why this might be, the reason for the fitness advantage is still unclear. Although feeding behavior itself has already been shown to be unrelated to fitness[15], we propose that differences in food consumption levels between *npr-1* alleles could be contributing to traits like sexual maturity timing and number of eggs, which in turn lead to the fitness advantage. If we find that *npr-1* leads to enhanced fitness as a result of increased food consumption, this could be a unique opportunity to examine the metabolic mechanisms through which a more active neuropeptide receptor may increase food consumption. Given the theorized links between fitness and genetic predispositions for obesity, this could contribute to obesity in humans as well. Thus, using optogenetic manipulation of *C. elegans* pharyngeal muscles, we aim to explore causative effects of food consumption on fitness as a result of differences in *npr-1* alleles. Through this process, we hope to provide insight into potential mechanisms salient to energy metabolism and genetic predispositions to obesity.

Literature Review

Obesity is an expanding public health burden known to contribute to life-threatening cardiovascular, metabolic, and endocrine disorders[17]. Because obesity is affected by complex interactions between environmental, cultural, social, and genetic factors, developing medical treatments and preventative programs is a top priority of research and social work. To achieve these ends, there has been significant interest in the study of metabolism, fat storage, feeding circuits, hormones, and feeding behavior. Currently in mammals, the role of leptin signaling in the hypothalamus has been one of the most studied processes involved in the moderation of feeding behavior. Leptin is a hormone encoded by the LEP gene and secreted by adipose fat tissue. The hormone was identified by a natural loss of function mutation which caused obesity in mice[18] and is now known to convey a satiety signal via Agouti-related Protein (AgRP) and Proopiomelanocortin (POMC) neurons in the hypothalamus[19,20]. Leptin therapy is available for humans with a congenital leptin deficit, but typical obese patients have a resistance to leptin, making the exploration of alternative treatments worthwhile[21]. This is just one instance of the challenging setbacks experienced in obesity research.

One way to begin exploring new treatments is to focus on other genes that are involved in the neural circuit for feeding and biological pathways controlling metabolism. Predicting which genes should be the focus of potential therapeutics is not a trivial process, but many elaborate theories center around evolutionary pressures that might provide insight into attractive candidate genes. For example, one theory proposes that there was positive selection for "thrifty" genes which increased energy storage during times of surplus[5]. Now that humans constantly experience an abundance of food, obesity could be the product of this tendency to enhance energy storage. Another theory suggests obesity could stem from the need for different resting metabolic rates in response to changes in climate associated with human migration[4]. While the evolutionary mechanism is debated, there seems to be agreement that fitness advantages in ancestral humans led to genetic predispositions for obesity today. Understanding the mechanisms underlying why obesity has become so prevalent in humans, whether the reason be energy storage, metabolic rates, or something else entirely, will help guide research into new, more targeted therapeutics.

Among the overwhelming number of genes to choose from, much research has been done in mice to demonstrate the effects of mutant RFamide neuropeptides and neuropeptide receptors on feeding, metabolism, and fat storage[22-24]. One notable example in the RFamide family is the mammalian neuropeptide Y (NPY) receptor[25]. NPY receptors could be a very interesting subject for studying obesity because NPY is related to a host of obesity-related processes such as adipose fat generation and thermogenesis[6], as well as feeding behavior via the AgRP/POMC neuronal circuit[7-10]. Found frequently throughout the nervous system, this complex signaling molecule has not only orexigenic effects but also roles in cardiovascular regulation, seizure and cognition, stress, and modulation of neuroendocrine systems[11-14]. The various roles of NPY would explain the connection between obesity and the various health concerns associated with it. The problem lies in identifying which of these processes may have a direct, causal effect on obesity phenotypes. We propose that exploring the fitness advantage conferred by different NPY receptor alleles will allow us to address this question. Here, we develop a way to optogenetically induce dietary restriction in *C. elegans* and quantify diminished food consumption using

a pre-established feeding assay[15]. In the future, using this optogenetic manipulation of the nematode worm *C. elegans* will allow us to determine what causes fitness differences between worms with different neuropeptide receptor alleles.

The study of obesity in model organism *C. elegans*

Conveniently, the simple nematode model organism *C. elegans* has a homolog of human NPY receptors, which is encoded by the *npr-1* gene. *C. elegans* has a short life span, small size, and fully mapped neural connectome, making it an ideal species with which to rapidly study neuroscience, genetics, and evolution in ways which are not feasible in mammals. Although there are more recent mammalian ancestors to humans, *C. elegans* has many genes homologous to those found in humans. Based on a past study of the KLF protein family in the context of fat metabolism[26], *C. elegans* has been proposed as a useful model with which to compare human metabolic regulation, a process intrinsically linked with obesity. Already, the species has also been used in a variety of studies surrounding the effects of aerotaxis, population density, and food sensing on lipid metabolic rates[27]. In addition to this, *C. elegans* has been used in studies examining the effects of diet (including nutrients and glucose) and dietary restriction without malnutrition on lifespan[28-30], tying into similar studies in mammals aimed at understanding food consumption and obesity[31,32]. Studies on the Ins/IGF-1 pathway, which is involved in dauer formation and food consumption, have successfully demonstrated the ability to extend *C. elegans* lifespan[33]. The connection here between food consumption and lifespan in a signal molecular pathway is very interesting in the context of obesity research.

The *npr-1* gene in particular has also been extensively characterized in *C. elegans*. Especially interesting is that a single mutation at position 215 in *npr-1* results in differential feeding behaviors, food consumption, and fitness levels between different strains of *C. elegans*[15,16,34], supporting interest in the connection between neuropeptide receptors and processes related to obesity. Previously we found that feeding behavior itself did not cause a fitness advantage[15]. Instead, we theorize that greater food consumption and resulting energy availability conferred by *npr-1* mutations could enhance fitness. Greater availability of energy could explain the extensive pleiotropic effects *npr-1* has on sexual maturity, sperm and egg production, and pheromone sensing[15]. Thus, our overarching goal is to determine the impact of enhanced food consumption on the fitness advantage observed between two naturally occurring *npr-1* alleles, which could provide a unique opportunity to examine the ways in which a mutant neuropeptide receptor may increase food consumption.

A deeper dive into the genes and neural circuits at hand

The *npr-1* gene and an associated globin protein gene, *glb-5*, were identified first through genetic analysis of mutants[16] and confirmed later using quantitative trait loci (QTL) analysis[35]. The ancestral *npr-1* allele results in “social” feeding behavior caused by aerotaxis. Social worms clump and aggregate at low oxygen concentrations and have lower fitness and food consumption levels in food-rich laboratory conditions. Meanwhile, a gain of function mutation in *npr-1* results in “solitary” feeding behavior caused by a lack of aerotaxis. Solitary worms will roam independently of oxygen gradient and have higher fitness

and food consumption. Here, we focus on CX12311 as the model social strain and N2 as the model solitary strain.

As illustrated in Figure 1 to the right, both NPR-1 and GLB-5 act in the RMG-URX neuronal aerotaxis circuit[36]. NPR-1 inhibits the RMG neuron, while GLB-5 is in the oxygen sensing neuron URX[35]. Importantly, the RMG interneuron acts as a hub between a variety of other chemosensory and motor neurons[37], suggesting many possible complex interactions between feeding behavior, aerotaxis, and other environmental cues. Indeed, while *npr-1* mutations clearly affect the aerotaxis circuit, there is a number of environmental conditions that will affect aerotaxis as well, such as population density[38], pheromones[37], noxious stimuli[39], and potentially even oxygen concentration preferences learned through experience[40]. Most of these have also been associated with other chemosensory neurons interacting with the RMG hub neuron. This is important to consider because, while we focus on food consumption, we also acknowledge the complexities of the aerotaxis and feeding circuitry in *C. elegans*. However, we maintain that food consumption and resulting energy availability would be crucial components in determining the effectiveness of other systems related to the RMG/URX neuronal circuit.

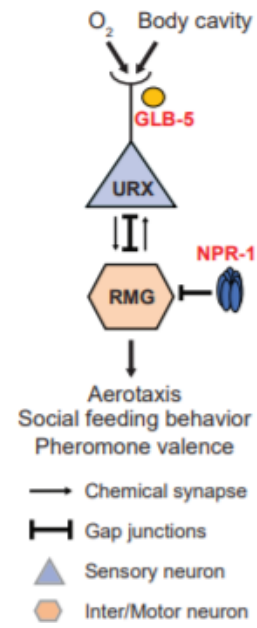

Figure 1: Diagram from Zhao et al (2018) illustrating the URX/RMG circuit

Our approach

Given the aforementioned pleiotropic effects of *npr-1* and *glb-5*, it is possible that the mutations in these genes have occurred due to some other physiological advantage they confer that is independent of feeding behavior. Based on the finding that the solitary allele of *npr-1* increases food consumption[15] and the extensive involvement of NPY in metabolic functions in mammals, we theorize that increased food consumption enhances fitness. Our hypothesis is further supported by another finding that intestinal fat storage affects sensory neuron URX resting activity, demonstrating a link between metabolism and the processing of oxygen concentration[41]. URX has been shown to be heavily involved in the altering of aerotaxis behaviors based on the sensation of oxygen concentration[37,42].

While much progress has been made in studying feeding, metabolism, and fat storage in *C. elegans*, there are two main aspects of previous research that we aim to build upon in order to evaluate the role of *npr-1*. First, past studies have used pharyngeal pumping rate as a metric for food consumption, but it appears that pumping rate does not necessarily correspond to how much food is consumed[15]. Instead, we propose using a feeding assay developed by Zhao et al (2018) to more accurately quantify food consumption. Second, past studies have used optogenetics to directly analyze diminished or enhanced function of specific neurons or muscles in *C. elegans*[43]. Optogenetics has also been used specifically within the aerotaxis circuit in pharyngeal muscles[44] and the RMG neuron[45]. We will use the red-shifted channelrhodopsin Chrimson[46] to inhibit pharyngeal muscles because red light is less phototoxic and penetrates the worm body better than blue light[43]. Combining the feeding assay with optogenetic inhibition of the pharynx will allow us to directly measure how reduced food intake may affect fitness.

Methodology

Materials

Strain/Reagent/Item	Source	Identifier
OP50 (*E. coli* strain)	Caenorhabditis genetics center (CGC)	RRID:WB-STRAIN: OP50
OP50 GFP (*E. coli* strain)	Caenorhabditis genetics center (CGC)	RRID:WB-STRAIN: OP50-GFP
N2 (*C. elegans* strain)	Cori Bargmann Lab (The Rockefeller University)	RRID:WB-STRAIN:N2
CX123121 (*C. elegans* strain)	PMID: 21849976	RRID:WB-STRAIN:CX12311
CX16904 (*C. elegans* strain)	PMID: 30580965	RRID:WB-STRAIN:CX16904
FUDR (Chemical compound, drug)	Sigma	Cat. No.: F0503
1x Antibiotic-Antimycotic (Chemical compound, drug)	ThermoFisher	Cat. No.: 15240062
All-trans retinal (Chemical compound, drug)	Sigma	Cat. No.: R2500
550nm MINTL5 LED (Equipment)	ThorLabs	Part No.: MINTD3
Aluminum Heatsink (Equipment)	Adafruit	Cat. No.: 3082
Screw Terminals 3.5mm Pitch (Equipment)	Sparkfun	Cat. No.: PRT-08084 ROHS
Wall Adapter Power Supply – 9VDC, 650mA (Equipment)	Sparkfun	Cat. No.: TOL-15314 ROHS
Metro Mini Micro Controller (Equipment)	Adafruit	Cat. No.: 2590
FemtoBuck LED Driver	Sparkfun	Cat. No.: COM-13716 ROHS

C. elegans Strains and Growth Conditions

C. elegans laboratory strain N2, wild strain CX12311, and optogenetic strain CX16904[44] were grown in the following standard conditions[47]. All worms were incubated at 20°C on nematode growth medium (NGM) agar plates, fed *Escherichia coli* (OP50), and transferred to new plates every four days[47]. Worms were allowed to grow for at least three generations in these conditions without starvation before any assays were performed.

Pharyngeal Pumping on ATR Lawns

Worms were grown in the dark overnight on agar plates seeded with OP50 or OP50 with 50µM all-trans retinal (ATR; Sigma-Aldrich R2500). Pumping rates were visualized using a dissecting microscope

for one minute and calculated based on the number of pumps per minute. The microscope's green light was used for illumination and pharyngeal inhibition as the wavelength was within the appropriate range to affect the rhodopsin[46].

Feeding Assay

Feeding assays were conducted as previously described by Zhao et al (2018) with slight modifications. To determine food consumption, worms were allowed to feed on OP50:GFP (pFPV25.1) for 18 hours. The fluorescent signal given by a Synergy H4 Biotek microplate reader before and after those 18 hours was used to quantify food consumption. Worms were synchronized with alkaline-bleach and washed three times with S Basal before being grown overnight in a tube roller. About 200 L1 stage worms of each strain were then placed on OP50-seeded NGM agar plates and incubated at 20°C for fifty hours. Meanwhile, 24-well plates were filled with 0.75 mL NGM agar containing 1X antibiotic-antimycotic mixture (ThermoFisher 15240-062), 1X kanamycin, and 25 µM FUDR.

Once the worms had grown to L4 stage after 50 hours, the 24-well plates were seeded with 20 µL OP50:GFP or 20 µL OP50:GFP containing 50 µM ATR. Ten worms were placed in each well containing cultured OD600 of 4.0 (CFU ~ 3.2910^9/mL) OP50:GFP. Four control wells were reserved to measure the natural decay in fluorescence of OP50:GFP. Because fluorescence readings can be slightly affected by the location of the well on the place, the placement of each condition was randomized for every experiment. Fluorescence measurements were made using area scanning protocol on a BioTek Synergy H4 multimode plate reader at 6 mm read height. Plates were then left at room temperature (approximately 20°C) for 18 hours in the dark, after which fluorescence readings were taken again using the same protocol.

Control wells contained OD600/mL = 4, 3, 2, or 1 OP50:GFP. These concentrations were used to define the relationship between bacteria concentration and fluorescent measurement f_{cntrl} at 0 and 18 hrs.

$$f_{cntrl}(OD600_{0hrs}) = m_{0hrs} * OD600_{0hrs} + b_{0hrs}$$

$$f_{cntrl}(OD600_{18hrs}) = m_{18hrs} * OD600_{18hrs} + b_{18hrs}$$

Once this was quantified, fluorescence values of both experimental and control wells at 0 and 18 hours (f_0 and f_{18}) could be translated into CFUs using the following relationship.

$$CFU = 8 * 10^8 * OD600$$

$$CFU_{0hrs} = 8 * 10^8 * OD600_{0hrs} = \frac{f_0(OD600_{0hrs}) - b_{0hrs}}{m_{0hrs}}$$

$$CFU_{18hrs} = 8 * 10^8 * OD600_{18hrs} = \frac{f_{18}(OD600_{18hrs}) - b_{18hrs}}{m_{18hrs}}$$

$$\Delta CFU = CFU_{18hrs} - CFU_{0hrs}$$

The above equations were used to normalize all wells. Statistical significance between strains was determined using the two-tailed Mann-Whitney U Test or two-way ANOVA with Tukey HSD post-hoc analysis.

LED Set-Up for Optogenetics in Feeding Assay

A ThorLabs MINTL5 LED (550 nm) was mounted to an Adafruit aluminum heat sink (ID3082) and positioned 4 cm away from the 24-well plate lid and aligned with the center of the plate. The power readings for the wells were greatest in the center but were high enough on the outer wells to produce pharyngeal inhibition in CX16904 worms. The LED was programmed using Arduino to alternate on and off.

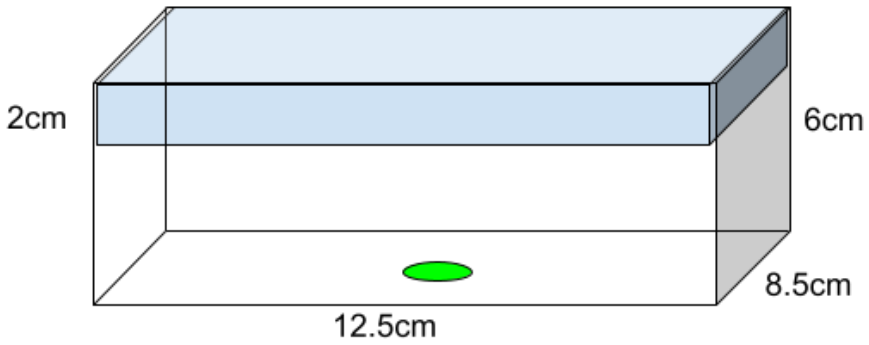

Figure 2: LED setup. The blue box towards the top represents the 24-well plate used in the feeding assay. The larger clear box was constructed using opaque black cardboard stock so that no other light could get in. We determined that having the light source (green circle) 4 cm away from the lid of the 24-well plate produced enough power to inhibit the CX16904 pharynx. During experiments the 24-well plate would be taped in place and the entire box turned upside-down so that the plates would not lose too much moisture.

Results

We first established our LED set-up and the characteristics of the optogenetic strain CX16904. Then we conducted feeding assays to determine food consumption of the control strain N2 compared to the optogenetic CX16904. CX16904 expresses the modified channelrhodopsin Chrimson in the pharyngeal muscles, and these muscles are contracted upon red or green light exposure. We wanted to see if we could decrease the food consumption of the optogenetic strain using LED exposure, starting with a 30s on/30s off cycle.

Characterizing the effects of ATR and LED exposure

Pumping rates were measured to confirm the functionality of the CX16904 optogenetic modification. We found that CX16904 pharyngeal pumping was indeed abolished by ATR consumption and LED exposure (Figure 3). All other strains had an average of between 200 and 250 pharyngeal pumps per minute, but CX16904 had no pumps per minute when exposed to ATR and LED. Additionally, ATR consumption and LED exposure resulted in prolonged pharyngeal inhibition over time (Figure 4). CX16904 exposed to ATR and LED has a strongly diminished pumping rate for at least 40 hours, which would satisfy the full 18 hours of pharyngeal inhibition required for the feeding assay.

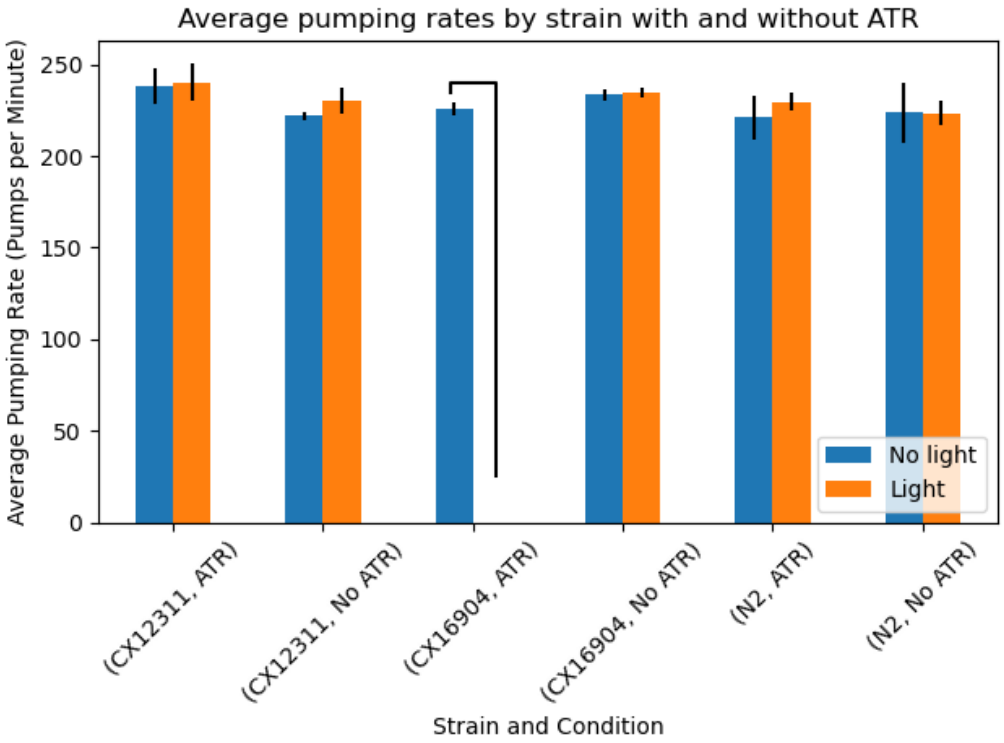

Figure 3: Average pumping rates per minute by strain with and without ATR. Pumping rates in the optogenetically modified strain CX16904 were abolished with ATR consumption and subsequent LED exposure (indicated by the black lines), while all other strains and conditions remained relatively consistent in pumping rate. Each bar represents three worms, error bars show standard error. Worms were plated and allowed to consume OP50 or OP50+ATR overnight before pumping measurements.

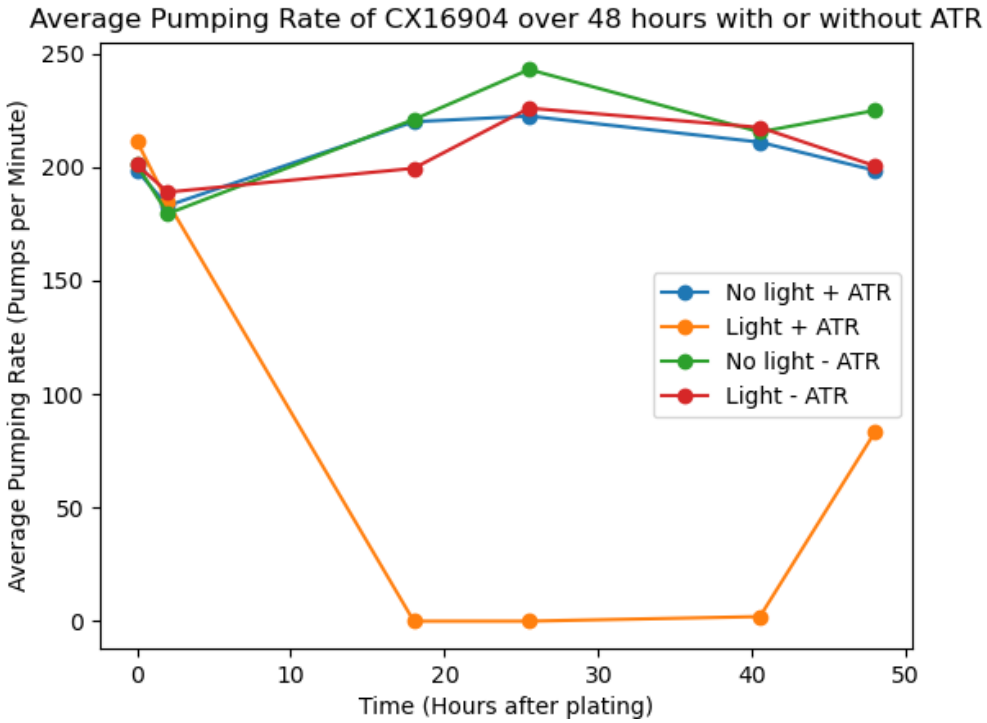

Figure 4: Average pumping rate per minute of CX16904 with and without ATR. CX16904 worms had relatively consistent food consumption over 48 hours, except for when they consumed ATR and were exposed to LED. Pumping rate started to recover in CX16904 worms with ATR and LED at 48hrs. Each dot represents the average pumping of two worms. Time point 0hr represents the pumping rates of the worms immediately after they were placed on the plate. In between measurements, all worms were kept in the dark at room temperature.

Food consumption

Without any optogenetic manipulation, N2 worms consumed more food than CX12311, which was expected based on previous experiments[15]. N2 worms also consumed more food than CX16904 worms ($p < 0.00001$), while CX16904 consumed more food than CX12311 ($p < 0.01$) (Figure 5). Once establishing this baseline level of food consumption for CX16904, we determined food consumption rates of N2 worms with and without ATR and a 30s on/30s off LED cycle. The presence of ATR in the OP50 reduced N2 food consumption ($p < 0.05$), but LED exposure alone did not significantly alter feeding behavior (Figure 6). Using two-way ANOVA, we also determined that there was no combined effect of ATR and LED exposure on food consumption, as expected. Subsequently, we repeated this experiment with both N2 and CX16904. Consistent with our findings on N2, ATR significantly diminished food consumption in both N2 ($p < 0.01$) and CX16904 ($p < 0.00001$) based on a two-way ANOVA (Figure 7). Interestingly, the 30s on/30s off LED exposure also seemed to have an effect on food consumption in N2 ($p < 0.01$) and CX16904 ($p < 0.01$). However, the interaction of LED and ATR did not significantly reduce N2 or CX16904 food consumption with the 30s on/30s off LED cycle. Because this LED cycle did not seem to effectively diminish CX16904 food consumption, we tried increasing the time that the LED was on to 90s (Figure 8). Again, ATR decreased food consumption in both N2 ($p < 0.0001$) and CX16904 ($p < 0.00001$), but this time the interaction between LED and ATR in CX16904 decreased food consumption in CX16904 ($p < 0.001$) but not N2.

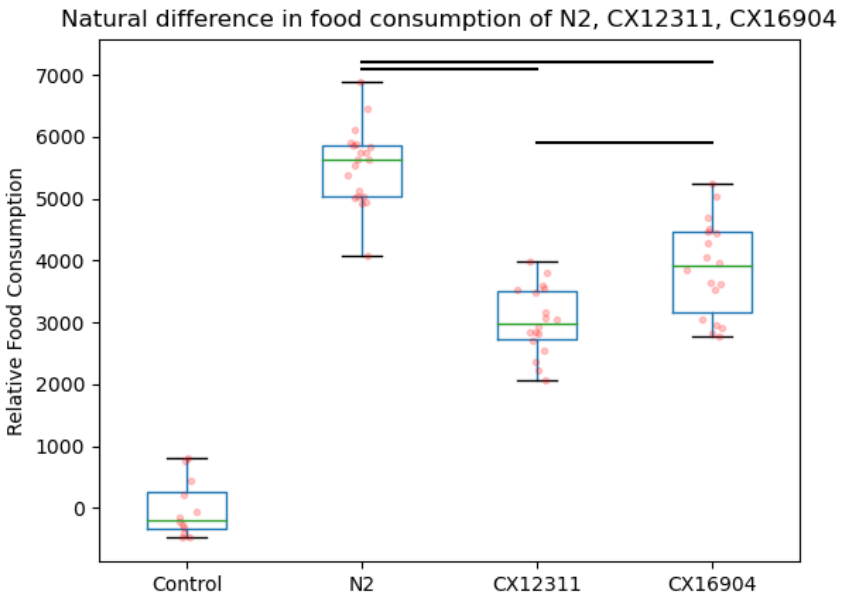

Figure 5: Natural Difference in Food Consumption of N2, CX12311, and CX16904. Control fluorescence was normalized using linear regression and worms were kept in a 20C incubator. Relative food consumption is in terms of difference in fluorescence readings. N2 had the greatest food consumption compared to CX12311 and CX16904 ($p < 0.00001$). CX16904 ate more than CX12311 ($p < 0.001$). Horizontal black lines indicate those condition pairs which were significantly different by two-tailed Mann Whitney U Test.

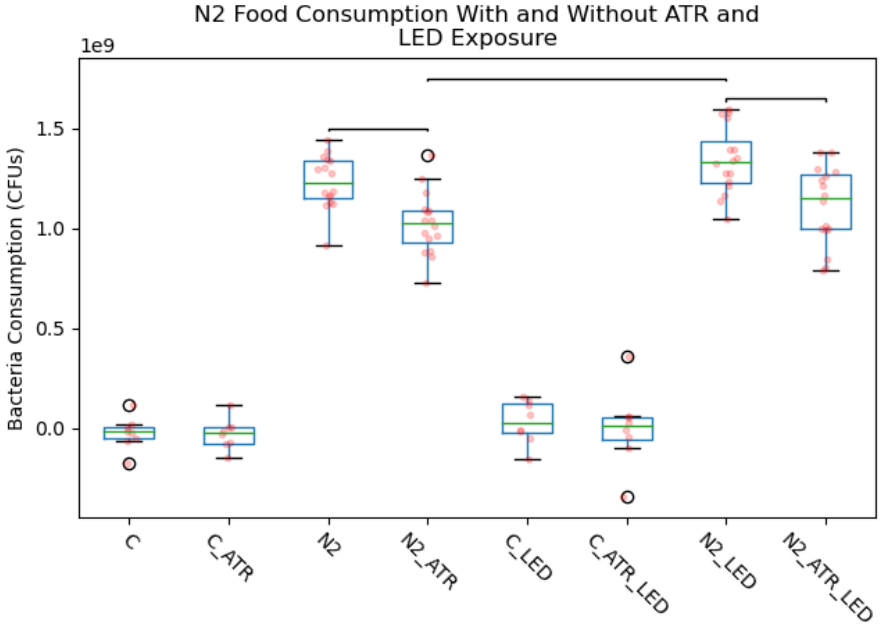

Figure 6: N2 Food Consumption with and without ATR and LED exposure. Animals were exposed to the LED half of the 18 hour time period, with a 30s on/30s off cycle. Bacteria consumption measured in colony forming units (CFUs) based on fluorescence readings. The food consumption of N2 worms diminishes when ATR is in the bacterial lawn. Horizontal black lines indicate those conditions which were significantly different ($p < 0.05$) by two-way ANOVA and post-hoc Tukey-HSD.

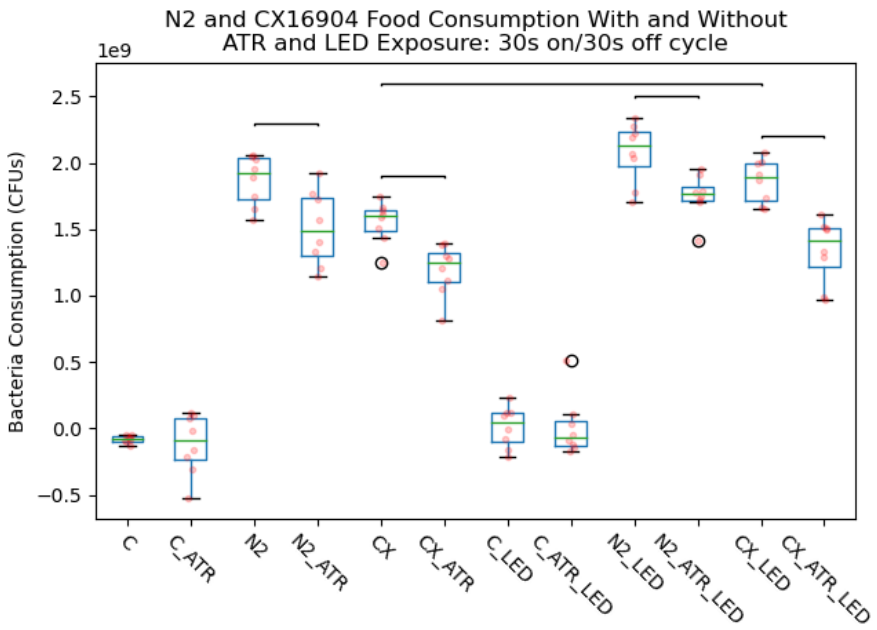

Figure 7: N2 and CX16904 Food Consumption with and without ATR and LED exposure. Bacteria consumption measured in colony forming units (CFUs) based on fluorescence readings. With a 30s on/30s off LED cycle, N2 was again unaffected by LED exposure, but had diminished food consumption when eating OP50 with ATR ($p < 0.05$). CX16904 also had diminished food consumption on ATR lawns ($p < 0.01$). CX16904 seemed to eat more food with LED exposure when ATR was not present ($p < 0.01$), but CX16904 food consumption was unaffected by the LED when ATR was present ($p = 0.19$). Horizontal black lines indicate those conditions which were significantly different ($p < 0.05$) by two-way ANOVA and post-hoc Tukey-HSD.

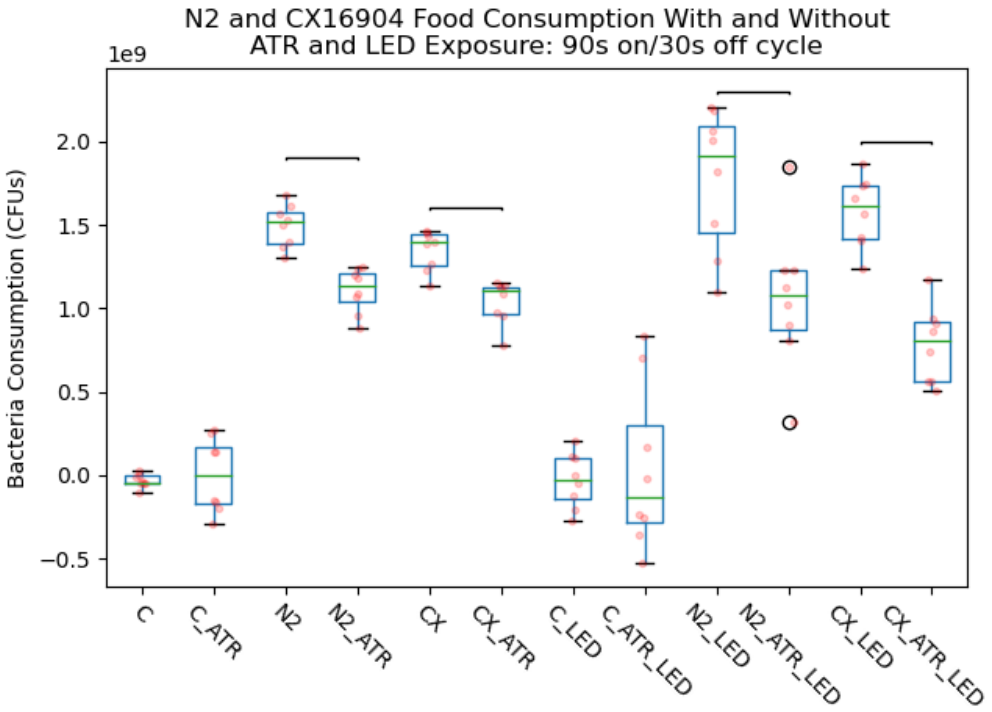

Figure 8: N2 and CX16904 Food Consumption with and without ATR and LED exposure. Bacteria consumption measured in colony forming units (CFUs) based on fluorescence readings. With a 90s on/30s off LED cycle, N2 and CX16904 were again not significantly affected by LED exposure, although the variation in the food consumption was greater. N2 and CX16904 had diminished food consumption when eating OP50 with ATR ($p < 0.0001$, $p < 0.00001$, respectively). CX16904 ate significantly less when exposed to both ATR and the LED ($p < 0.001$). Horizontal black lines indicate those conditions which were significantly different ($p < 0.05$) by two-way ANOVA and post-hoc Tukey-HSD.

Discussion

Considering the theorized links between fitness and genetic predispositions for obesity, *npr-1* and *glb-5* could have direct applications to the study of obesity in humans. Our findings establish a foundation for further studying the effects of optogenetic manipulation of food consumption on lifespan and evolutionary fitness in *C. elegans*. Here, we present preliminary findings characterizing food consumption of the optogenetically modified CX16904 which will benefit future research on the effect of food consumption on lifespan and evolutionary fitness. Importantly, we found that CX16904 worms naturally ate less than N2 worms whether or not they fed on ATR lawns ($p < 0.00001$), suggesting the optogenetic construct may have an immediate effect on food consumption because otherwise N2 and CX16904 have the same alleles of *npr-1* and *glb-5*. Altering protein expression in any cells can affect cell health and electrophysiology in potentially unexpected ways[48], and this could be the case for CX16904.

Additionally, both N2 and CX16904 showed diminished food consumption in the presence of ATR (Figure 6,7). ATR is usually considered to be a standard co-factor fed to *C. elegans* because it is not naturally produced in *C. elegans* as it is in mammals[49,50]. However, our findings suggests that ATR itself has an effect on the worms' food consumption, which has not been previously reported. The presence of attractants and repellants in the environment of *C. elegans* can alter pumping rate[51], which would be one potential explanation of our observations. Additionally, altering the environment of *C. elegans* could be unexpectedly altering *C. elegans* physiology, behavior, or metabolism, any of which could also be affecting food consumption in unexplained ways. Further investigation of the effects of ATR on *C. elegans* may be valuable for future optogenetics experiments.

While worms exposed to the LED cycle without ATR did not have significantly different food consumption levels, the variation in the data seemed to increase, potentially as a result of natural light- or temperature-induced changes in behavior[43]. Bright, low-wavelength light exposure has been previously shown to naturally lead to accelerated locomotion[52] and inhibited feeding[53] in *C. elegans* via the endogenous photoreceptor LITE-1. Our results may differ slightly because we used green light, which has a longer wavelength. Additionally, the inhibition of feeding was quantified using pharyngeal pumping[53], which may not accurately reflect true food consumption as in our assay.

When the CX16904 strain fed on ATR lawns and was exposed to a 30s on/30s off LED cycle for 18 hours, their food consumption did not seem to be significantly altered, even when accounting for the ATR-induced change in feeding. This could be explained by some mechanisms of homeostasis in the worms. It is possible that *C. elegans* already only eat about half of the time they are on bacterial lawns, so intentionally inhibiting CX16904 for half of the 18 hours would have no effect because the worms would just each when the LED was off. Alternatively, it is possible that the worms may eat at a standard rate when not exposed to the LED. Potentially, CX16904 compensates for being inhibited by the LED by eating more or faster than normal when the LED is off. However, using a 90s on/30 off LED cycle during the feeding assay did decrease CX16904 food consumption ($p < 0.001$). We will want to further examine different cycles to see if we can reduce food consumption in CX16904 to an even greater extent.

Once we determine the optimal LED cycle, we can focus on lifespan assays and eventually fitness assays. If we find that *npr-1* leads to enhanced fitness as a result of increased food consumption, this could

be a unique opportunity to examine the pathways through which a more active neuropeptide receptor may increase food consumption. Considering the theorized links between fitness and genetic predispositions for obesity, these genes could have direct applications to the study of obesity in humans. In the future, we aim to explore causative connections between food consumption and fitness as a result of differences in *npr-1* and *glb-5* alleles. Aside from analyzing *npr-1*, it would also be interesting to determine the lifespan of worms with reduced food consumption compared to *daf-2* mutants, which have twice the lifespan compared to wild type worms[33]. *daf-2* is part of the Ins/IGF-1 signaling pathway, which is itself related to dauer formation, food consumption, and longevity, making it also salient to obesity research. Through this process, we hope to provide insight into potential mechanisms salient to energy metabolism and genetic predispositions to obesity.

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